Review



# Aged Garlic Extract and Its Bioactive Molecules S-Ally-Cysteine and S1-Propenl-Cysteine: A Review Focusing on Evidences Sup-Porting Their Use for Mitigating the Effects of Cigarette Smoking

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# Aged Garlic Extract and Its Bioactive Molecules S-Ally-Cysteine and S1-Propenl-Cysteine: A Review Focusing on Evidences Supporting Their Use for Mitigating the Effects of Cigarette Smoking

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**Abstract**

One of the major social issues worldwide is the tobacco dependency and the cigarette smoking (CS) abuse. Given the significant impact of cigarette smoking on human health and disease, extensive tobacco use and cigarette smoking abuse are certainly a form of drug addiction and should be considered a serious threat to human health. Notably, healthcare spending attributable to cigarette smoking is very high. In this regard, a significant number of biomolecules of natural origin have been described as capable of mitigating the adverse effects of cigarette smoking. In this review (a) we discuss the impact that the habit of smoking tobacco has on human health and (b) we describe products of natural origin capable of mitigating the cigarette smoke effects. The conclusion of this review article is that the available information strongly sustains a possible use of the anti-inflammatory aged garlic extract (AGE) and its bioactive components for mitigating the detrimental effects of cigarette smoke on human tissues. The key reasons for proposing this application are that AGE and its key components are potent anti-inflammatory agents, bind Toll-like Receptor-4, inhibit Nuclear Factor-κB, inhibit the expression of pro-inflammatory genes, revert apoptosis induced by cigarette smoke in several cellular model systems and are strong inhibitors of Reactive Oxygen Species (ROS) formation.

**Keywords:** natural products; cigarette smoke; inflammation; aged garlic extract

## 1. Introduction

One of the major social issues worldwide is the tobacco dependency and the cigarette smoking (CS) abuse [1]. According to the "WHO global report on trends in prevalence of tobacco use 2000–2030" (16 January 2024, ISBN: 978-92-4-008828-3) [1], although the total number of tobacco users has constantly decreased considering the period 2000-2022, this number is still expected to be very high (around 1.20 billion) by 2030 [1,2]. Given the significant impact of cigarette smoking on human health and disease [3–9], extensive tobacco use and cigarette smoking abuse certainly are form of drug addiction and should be considered a serious threat to human health [1]. Notably, healthcare spending attributable to cigarette smoking is very high [10,11]. In order to limit the tobacco use, several actions have been considered to help quit smoking [12–15], such as bans of tobacco advertising [16] and introduction of taxes as a share of cigarette price [17,18]. Despite these initiatives, the habit of smoking tobacco on a consistent basis is still a very significant social problem. In this regard, a significant number of biomolecules of natural origin have been described as capable of mitigating the adverse effects of cigarette smoking "in vitro" on cells and tissues and "in vivo" on complex organisms [19–21]. In this review (a) we discuss the impact that the habit of smoking tobacco has on health and the costs for national health systems, (b) we will describe products of natural origin capable of mitigating the adverse effects of cigarette smoking and (c) we will focus our attention on

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the possible use of aged garlic extract (AGE) and its bioactive components to mitigating the adverse effects of cigarette smoking.

## 2. Impact of Cigarette Smoke on Human Health

### *2.1. Smoking and Human Diseases*

Smoking causes cancer [22], heart disease [23], stroke [24], lung diseases [9,25], diabetes [26], chronic obstructive pulmonary disease (COPD) [27], and pancreatic diseases [28], as shown in Figure 1. Smoking is a particularly large problem in high-income countries, where preventable diseases and deaths are mainly caused by cigarette smoking [29]. The impact of smoking at the individual level is devastating, considering that the life expectancy of regular smokers is believed to be about 10 years lower than that of non-smokers. [29]. Reducing the number of cigarette smokers could be achieved through the activation of global health campaigns, including the ban on tobacco advertising, the introduction of taxes on cigarettes, the development of plans to help people quitting smoke. All these issues are discussed by Roser (https://ourworldindata.org/smoking-big-problem-in-brief#) [Accessed on May 22, 2025] [29]. For example, many governments have made cigarettes significantly more expensive through a process of incremental taxation. This intervention, causing a reduction in the affordability of cigarettes, is one of the most important, and cost-effective, ways to reduce smoking and improve public health [29].

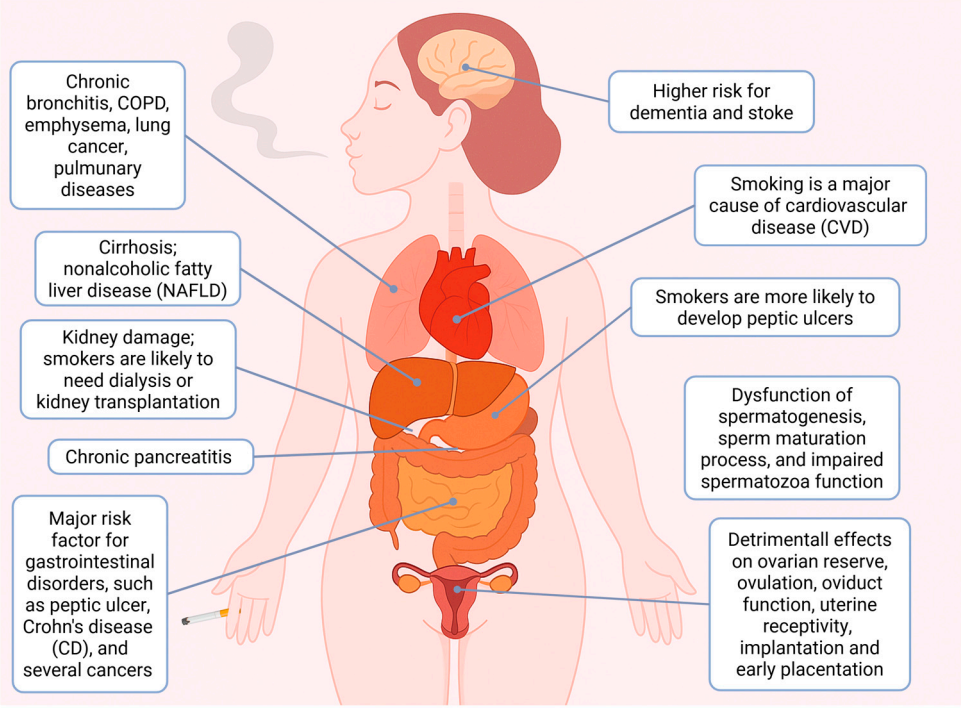


**Figure 1.** Human pathologies associate to cigarette smoking abuse. Picture created using Bio-Render.com (July 16, 2025).

### *2.2. Smoking and Cancer*

Smoking (and indirect smoking) causes or increases the risk for many types of cancer [30–32], including acute myeloid leukemia [33], bladder cancer [34], cervical cancer [35], colorectal cancer [36], esophageal cancer [37], prostate cancer [38], kidney cancer [39], laryngeal cancer and other throat cancers [40], liver cancer [41], lung cancer [42], oral cancer [43], pancreatic cancer [44] and stomach cancer [45].

In this respect, it should be underlined that tobacco smoking is associated in many cases with reduced efficacy or even failure of first-line cancer treatments; this causes incremental costs of the

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management of cancer patients [46]. In this respect, it is generally accepted the notion that smoking seriously impacts of on health system costs, including those regarding the cancer patients [47–51]. Accordingly, it is imperative that more stringent steps are taken to reduce the huge economic burden of human pathologies (including cancer) linked to smoking.

## 3. Mechanism(s) of Action of Cigarette Smoking: Inflammation

The cellular and molecular mechanisms responsible for the solid interplay between cigarette smoke (CS) and inflammation have been reviewed by Lee et al. [52]. In this respect, it should be underlined that the identification of cellular, biochemical and molecular effects of CS is a key step for the identification of molecular targets for medical interventions. As a first consideration, we should mention that the several toxins and the trace amounts of microbial cell components present in CS induce chronic inflammation [53–55]. In the CS dependent activation of pro-inflammatory genes, several proteins play a crucial role and should be considered as possible biochemical target for therapeutic intervention, among which the Nuclear-factor kB (NF-kB) pathway [56,57], associated with the activation of Toll-like receptor 4 (TLR4) [58–63].

Several experimental model systems are available to characterize the effects of cigarette smoke on cultured cell lines and the mitigation of these detrimental effects using natural products. Two are based on the production of "Cigarette Smoke Condensates" (CSCs) [64–66] and of "Cigarette Smoke Extracts" (CSEs) [67–69] (Figure 2).

Figure 2 reports a pictorial representation of the production of CSC and CSE starting from cigarette burning.

The key step of CSC preparation is the trapping of the cigarette condensate in a 0.22 μm filter pad; then the cigarette smoke particulates are eluted using solvents, such as methanol, dimethyl sulfoxide (DMSO) or ethanol, recovered and transferred to tissue culture medium (CSC) for testing the CSC effects on cultured cells. Description of CSC preparation methods can be found in Kim & Kim [70] and in Mathewson [71]. CSE is an aqueous solution that contains toxic compounds produced by cigarette smoke. Therefore, CSE is useful to determine the effects of cigarette smoke on in vitro cultured cell lines. CSE can be prepared by collecting the smoke from a cigarette as shown in Figure 2. The cigarette smoke is "bubbled" in cell culture medium under a negative pressure generated by a peristaltic pump. The aqueous components are therefore diluted in the cell culture medium, that, at the end of the procedure, is referred as "Cigarette Smoke Extract (CSE). The parameters to be considered are the following: (a) number of the cigarettes; (b) volume of the cell culture medium and (c) flow rate generated by the peristaltic pump. Description of CSE preparation methods can be found in Amel Al-Hashimi et al. [69], Agraval et al. [72], Higashi et al. [73] and Wight [74]. A detailed protocol is available (https://dx.doi.org/10.17504/protocols.io.bnymmfu6; accessed on May 22, 2025).

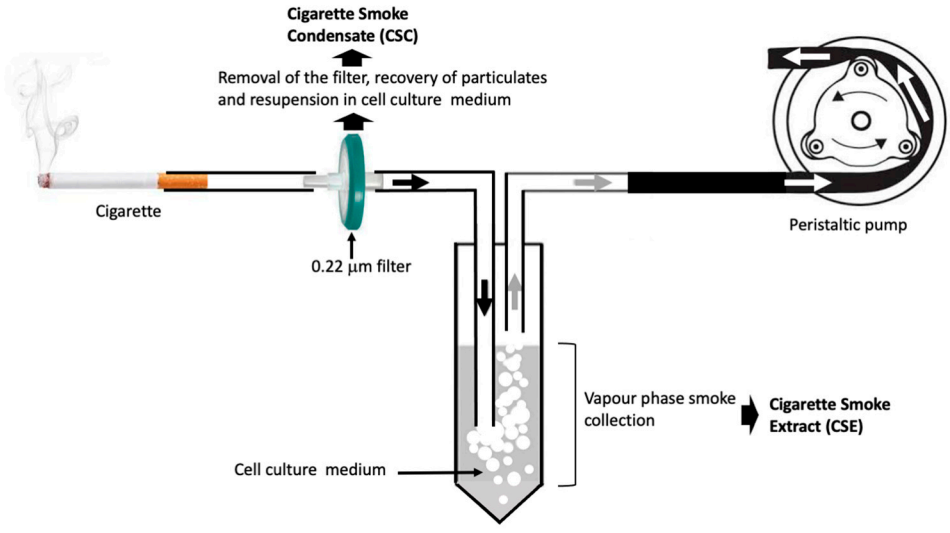

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**Figure 2.** Scheme outlining the reparation of "Cigarette Smoke Concentrates" (CSCs) and "Cigarette Smoke Extracts" (CSEs), using information taken from Kim and Kim (2023) [70], Higashi et al. (2014) [73] and Wright (2015)[74].

In addition of using CSC and CSE, the effect of cigarette smoke in vitro can be assessed by direct exposure of cells or cellular tissues to cigarette smoke based on the air-liquid interface exposure [75]. In this respect, Singh et al. presented a perspective view of the challenges and opportunities of "Lung-on-Chip" technologies in studies focusing on cigarette smoking related in vitro inhalation toxicology [76]. With respect to chemical composition of CSC and CSE, several studies are available [77–81]. In this respect, Kim et al. compared the volatile organic compounds (VOCs) of cigarette smoke condensate (CSC) and extract (CSE) samples [82]. CSC sample mainly contained nicotine, nicotyrine and lower relative amount of 1,2,3-propanetriol, triacetate, ethyl chloride and phenol [82]. The main composition of the CSE sample was different and contained acetonitrile, acetone, 2-hydroxy-2-methyl-propanenitrile, and lower amounts of nicotine and nicotyrine [82]. Therefore, considering that the compounds in CSC and CSE are different, the effects (including toxicity) determined using the CSC and CSE might differ. The following chapters summarize the effects of CSC and CSE on biological functions, most of which related to inflammation.

### *3.1. Cigarette Smoking and Nuclear Factor-kB (NF-kB)*

Concerning the effects of cigarette smoking on the NF-kB pathway, Anto et al. found that the CS condensate mediated induction of cyclooxygenase-2 was associated with activation of NF-kB through phosphorylation and degradation of IkappaB(alpha) [56]. The proteasome-linked degradation of IkappaB(alpha) causes the translocation of NF-kB to the nucleus and the transcriptional activation of NF-kB dependent genes [83–87]. Activation of NF-kB by cigarette smoke was also reported by Zhang et al. [88] and by Wang et al. [89]. Accordingly, products from natural world targeting the NF-kB signaling pathway are of great interest and should be considered as potential anti-inflammatory agents for mitigating the effects of cigarette smoking [90–93]. For instance, Wang et al. reported that ghrelin inhibits interleukin-6 production induced by a Cigarette Smoke Extract (CSE) and this inhibition is based on targeting the NF-kB pathway and [90]. In our own laboratory, we found that the NF-kB inhibitor corilagin attenuates the loss of cellular junctions induced by cigarette smoke in epithelial lung cells [93].

### *3.2. Cigarette Smoke and Toll-like Receptor-4 (TLR4)*

Nadigel et al. have reported that cigarette smoke increases TLR4 and TLR9 expression, thereby inducing increased cytokine production [61]. Interestingly, increased TLR4 expression was found in tissues of mice exposed to acute levels of cigarette smoke, and this was associated with lung inflammation [91,94]. Notably, elevated TLR4 and MMP-1 levels were found in lungs from smokers [94]. In conclusion, there is a general agreement on the fact that cigarette smoking related effects are mediated by activation of TLR-4 [58–63,94]. Accordingly, TLR4 inhibitors are expected to attenuate the acute cigarette smoke-induced pulmonary inflammation [94,95]. As a representative and informative example, the TLR4 inhibitor TAK-242 (resatorvid), that was studied by Wang et al. [91], who administered to mice exposed to cigarettes smoke. TAK-242 is a cyclohexane selected for inhibition of TLR4 [96]. It binds to the cysteine residue 747, preventing TLR4 binding with the toll-interleukin-1 receptor (TIR) domain-containing adaptor protein (TIRAP) [97] and downstream signal transduction. In the study by Wang et al. it was found very effective in mitigating the effects of exposure of mice to cigarette smoking. In fact, TAK-242 significantly decreased the accumulation of macrophages, neutrophils, lymphocytes and dendritic cells, and the upregulation of IL-6, IL-8 and TNF-$\alpha$ in BAL fluid and lungs of the cigarette smoke exposed mice [91]. The results of this study demonstrated that the release of various inflammatory mediators is inhibited by TAK-242; Notably, TAK-242 suppressed in lungs the expression of TLR4, MyD88 and the activation of NF-κB [91]. These findings support the concept that TAK-242-mediated inhibition of cigarettes smoke effects is associate

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to alterations of the TLR4/NF-κB signal pathway. Accordingly, TAK-242 can be proposed as a potent therapeutic agent in the treatment of cigarette smoke induced-pulmonary inflammation.

### *3.3. Cigarette Smoke and Increased Release of Pro-Inflammatory Proteins*

Fully in agreement with the effects of Cigarette smoke on the TLR/NF-kB axis (see chapters 3.1 and 3.2), cigarette smoke regulates the production of pro-inflammatory cytokines and chemokines by several in vitro cellular model systems [53,60,98–103]. Induced pro-inflammatory proteins include IL-6, TNF-α, IL-1β, IL-8, G-CSF, GM-CSF, MCP-1. For instance, Mio et al. reported that cigarette smoke induces IL-8 release from human bronchial epithelial cells [98]. Remarkably, cigarette smoke induced the expression of IL-8, but inhibits the release of eotaxin and RANTES release from airway smooth muscle [104].

### *3.4. Cigarette Smoke and Apoptosis*

Several reports are available on the induction of apoptosis with tobacco smoke and related products. Ramage et al studies the induction of apoptosis using A549 lung epithelial cells as in vitro model system [105]. In this study, A549 cells were treated with Tobacco smoke condensate and apoptosis was detected by DAPI staining. In addition, activation of Bax-alpha, an early event in the apoptotic process, was measured; the results demonstrated that tobacco smoke was able to initiates apoptosis in A549 airway epithelial cells and this resulted in a cell detachment and full apoptosis. Cigarette smoke induced apoptosis was also demonstrated in alveolar epithelial cells [106], endothelial cells [107,108] and Raw264.7 cells [109]. Concerning cigarette smoke induced apoptosis, Banerjee et al reported the very interesting observation that it was prevented by black tea in a guinea pig “in vivo” model system, associated with prevention of lung damage [110].

### *3.5. Cigarette Smoke Induced Formation of Reactive Oxygen Species (ROS)*

Cigarette smoke (CS) promotes ROS formation in different ways [111,112]. First of all, ROS, as well as radicals, are intrinsically present in CS [113–115]. In addition, CS constituents generate ROS through chemical reactions with biomolecules (quinones, redox-active metals, peroxy acids). For example, benzosemiquinones can penetrate the blood–air barrier, gaining access to the blood circulation, and consistently producing superoxide through quinone redox cycling, forming adducts with biomolecules, such as hemoglobin and albumin [116,117]. Furthermore, CS stimulates cellular ROS sources (NOX, mitochondria, uncoupled eNOS) to enhance ROS production [112,118]. Finally, CS components (such as ethyl vinyl chetone, chrotonaldehyde, acrolein) disrupts the antioxidant system, aggravating ROS generation and functions [112,119,120].

## 4. Natural Products for the Mitigation of Toxic Biological Effects of Cigarette Smoke

The impact of natural products in preventing some of the more common detrimental effects of cigarette smoke is very high, due to the low cost of these medical interventions, thereby allowing their use in developing low-incom countries. A comprehensive review focusing on the protective effects of medicinal plants against cigarette smoke has been published by Tabeshpour et al. [19]. In this respect, Oriola and Oyedeji reviewed plant-derived natural products as useful agents against common respiratory diseases caused by cigarette smoke [121] (see Figure 1). In this section we will discuss some of the available examples showing the validated use of natural products for protecting cells or tissue against cigarette smoking.

### *4.1. Silymarin*

Silymarin is a flavonolignan extracted from *Silybum marianum* (milk thistle seeds) reported to exhibit a broad spectrum of biological and pharmacological properties, including antioxidant, antiviral, anticancer, and immunomodulatory activities [122]. Li et al have reported that, in human

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bronchial epithelial cells "in vitro", silymarin attenuates inflammation induced by cigarette smoke extract; this effect was found to be based on the simultaneous inhibition of autophagy and ERK/p38 MAPK pathway [122]. In another study the effects of silymarin were analyzed "in vivo", demonstrating silymarin as a powerful inhibitor of airway inflammation induced by cigarette smoke in mice [123]. Silymarin pretreatment strongly reduced the secretion of TNF-$\alpha$, IL-1$\beta$, and IL-8 in BALF. These results suggest that silymarin attenuated both inflammation and oxidative stress induced by cigarette smoke. The anti-inflammatory effects reported in this study have been also suggested to be based, at least in part, on alteration of the mitogen-activated protein kinases (MAPK) pathway [122].

### *4.2. Eucalyptol*

1,8-cineole (Eucalyptol), a naturally occurring compound derived from botanical sources such as *Eucalyptus globulus*, *Rosmarinus officinalis*, and Camphor laurel (*Cinnamomum camphora*), has been extensively used in traditional medicine, exhibiting several biological properties, including anti-inflammatory, antioxidant, antimicrobial, bronchodilatoryand analgesic effects [124]. Recent evidence has also indicated its potential role in managing conditions such as Alzheimer's disease, neuropathic pain, and cancer [125]. Eucalyptol suppresses lipopolysaccharide (LPS)-induced production of proinflammatory cytokine through an action on NF-κB, TNF-$\alpha$, IL-1$\beta$, and IL-6 and the extracellular signal-regulated kinase (ERK) pathway [125]. Eucalyptol was found to modulate cigarette smoke extract-induced human bronchial epithelial cell damage [126]. Accordingly, Yu et al. reported that treatment with eucalyptol of rats exposed to cigarette smoke (CS) mitigates CS-induced lung injury through suppressing ICAM-1 gene expression [127]. In addition, Kennedy-Feitosa et al. reported that eucalyptol inhibits lung inflammation and oxidative stress and promotes lung repair in mice following cigarette smoke-induced emphysema [21,128].

### *4.3. Curcumin*

Curcumin is an important constituent of turmeric, extensively used in the traditional medicine [129,130]. The interest in the therapeutic potential of turmeric and the relative easy isolation of curcuminoids has led to their extensive investigation [130]. A comprehensive review on the protective effects of curcumin against cigarette smoke-induced toxicity is available [131], and research articles reported that curcumin and liposomal curcumin inhibit cigarette smoke induced senescence and inflammation in human bronchial epithelial cells [132]. This effect is associated with reduction of the expression of cigarette smoke extract-induced inflammatory markers IL-8 and IL-24 in vitro [133] through the modulation the PPARγ-NF-κB signaling pathway [134].

### *4.4. Taraxasterol*

The pentacyclic-triterpene Taraxasterol is extracted from *Taraxacum officinalis*, and exhibits anti-inflammatory properties [135]. Using lipopolysaccharides (LPS) -stimulated RAW264.7 cell as experimental model system, Taraxasterol was reported suppressing inflammatory cytokines, COX-2, and iNOS expression [136]. Xueshibojie et al. reported that Taraxasterol inhibits cigarette smoke-induced lung inflammation; ROS generation, IL-8 production, NF-κB activation, and TLR4 recruitment into lipid rafts were all CS-induced and inhibited by taraxasterol [137].

### *4.5. Sulforaphane*

Sulforaphane (SFN) is an isothiocyanate that is one of the most abundant bioactive components of Brassicaceae plants (such as broccoli) [138]. Previous studies have reported the antioxidant, antimicrobial, neuroprotective, cardioprotective, and anti-inflammatory activities of SFN [139]. Robust evidence concurrently indicates that the anti-inflammatory activity of SFN is based on NF-κB inhibition [140,141]. Published research results are available demonstrating that sulforaphane protects alveolar epithelial cells against injury caused by cigarette smoke extract and, in a first report,

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sulforaphane was demonstrated to inhibit, in human epithelial cells treated with cigarette smoke extracts, de novo synthesis of IL-8 and MCP-1 [142]. In another study, SFN was found to exhibit a protective role on alveolar epithelial cells exposed to cigarette smoke extract through an increase of Nrf2 expression [143,144].

### *4.6. Corilagin*

The polyphenol Corilagin is a member of the tannin family and it is extracted from different plants, including *Phyllanthus urinaria* [145], *Dimocarpus longan* [146] and *Geranium thunbergii* [147]. This natural compound has been reported to have beneficial effects in several cardiovascular disorders, hypertension, thrombosis, or atherosclerosis [145]. Zhao et al. have demonstrated that the anti-inflammatory properties of Corilagin is associated with a block of NFκB activation and NFκB nuclear translocation [148]. In addition, Corilagin decreases the production of proinflammatory proteins, such as TNF-$\alpha$, IL-1$\beta$, IL-6, IL-8, iNOS, and COX-2 [148]. Furthermore, Corilagin inhibits ROS production from leukocytes as well as formation of free radicals and lipid peroxidation in mitochondria [149,150]. In the study by Muresan et al. Corilagin was found mitigate the loss of cellular junctions induced in epithelial lung cells by cigarette smoke [93]. For their purpose, they have used Calu-3 cell line grown in air-liquid interface. The results of this study indicated that CS treatment is associated with a loss of cellular junctions in lung epithelium, possibly as a consequence of Cx-4HNE adducts formation; these CS induced alterations were found to be abolished by Corilagin [93].

### *4.7. Trans-4,4'-dihydroxystilbene*

Trans-4,4'-dihydroxystilbene (DHS) is an analogue of the naturally occurring hydroxystilbene resveratrol, present in grape skins, grape juices and red wines. These molecules are widely accepted as very interesting because of their diverse pharmacological attributes [151]. Wang et al. found that 4,4'-dihydroxystilbene ameliorates cigarette smoke-induced progression of chronic obstructive pulmonary disease by inhibiting oxidative stress and inflammatory response [152]. This study demonstrated that DHS is able to attenuate the CS-induced pulmonary alterations by targeting Nrf2 and NF-κB in vitro and in vivo, and could be developed as a preventive agent against pulmonary impairments induced by CS [152].

### *4.8. Other Example of Natural Products Against CS Effects*

Several studies support the concept that natural products from medicinal plants alleviate cigarette smoke-induced acute lung injury. Here are some examples. Liaqat et al. demonstrated that *Lavandula stoechas* significantly alleviates cigarette smoke-induced acute lung injury via modulation of oxidative stress and the NF-κB pathway [153]. Similarly, Hussain et al. found that *Cichorium intybus L.* significantly alleviates cigarette smoke-induced effects by lowering NF-κB pathway activation and inflammatory mediators [154]. Inhibition of the NF-kB pathway was also demonstrated as the mechanism of action explaining the anti-inflammatory and anti-oxidant properties of *Ipomoea nil (Linn.)* roth [155]. Furthermore, examples of reversion of the detrimental effects of cigarette smoke were found using propolis [156], mate tea [157] and grape skin extracts [158].

## 5. Aged Garlic Extract and Its Bioactive Components: Candidates for Mitigating the Cigarette Smoking Effects

Among a large variety of natural products of biomedical relevance, garlic-based products have recently gained great attention [159,160]. Among these products, AGE (aged garlic extract) is well known and its activity analyzed in detail [161]. AGE is a commercially available preparation obtained by immersing fresh garlic in 15% aqueous ethanol solution over a prolonged period of time (up to 20

months) at room temperature [161–165]. This natural product has been shown to exhibit immunomodulatory and anticancer properties [160,161].

The chemical composition of garlic and AGE has been described by Kodera et al. [166], Borek [167], Ryu et al. [168], El-Saadony et al. [169]. The beneficial effects of garlic have been attributed to several bioactive compounds, including the lipid-soluble allyl sulfur compounds (e.g., diallyl sulfide, diallyl disulfide and diallyl trisulfide) and water-soluble compounds, such as S-allyl-cysteine (SAC), S-allylmercaptocysteine (SAMC) and S-1-propenylcysteine (S1PC) [162–166]. In particular, water-soluble compounds (such as SAC and S1PC) are of interest, considering their high oral bioavailability, favorable pharmacokinetics and tissue distribution, which facilitate their clinical applications [170]. In this review, among the variety of chemical components [166], we focused on SAC and S1PC. These bioactive compounds might be extracted from AGE by unique manufacturing processes [165].

The anti-inflammatory Aged Garlic Extract (AGE) and its major bioactive components might be of great interest for mitigating the effects of cigarette smoking. The key reasons for proposing this application are summarized in Figure 3.

Notably, CS has been shown to induce a chronic inflammation. In this respect, several studies have revealed that AGE and its key components are potent anti-inflammatory agents, both “in vitro” and “in vivo” [171]. Furthermore, CS induced the TLR4/NF-kB pathway (se chapter 3.1 and 3.2). In this respect the AGE component S-allyl-l-cysteine (SAC) and S1-propenyl-l-cysteine bind TLR4 [172–174] and inhibit NF-kB [175].

A further consideration concerns the effects of CS on the expression of pro-inflammatory genes. CS induces IL-6, IL-8, IL-1β and several pro-inflammatory genes [60,98–104].

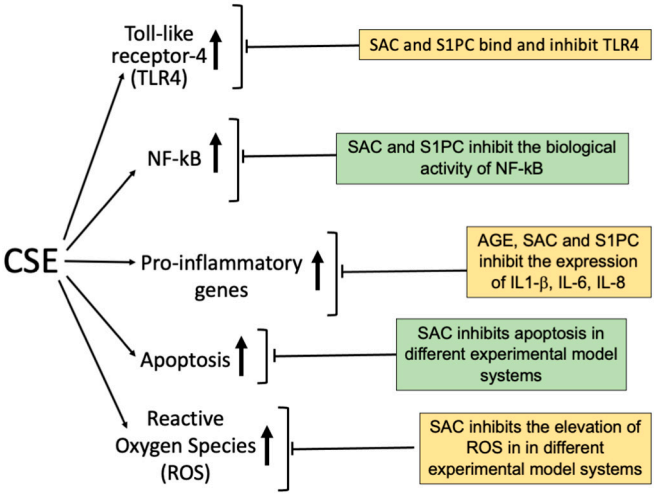


**Figure 3.** Biological features of AGE and AGE constituents SAC and S1PC supporting their use for mitigating the effects of cigarette smoke.

We and several other research groups have clearly shown that AGE and AGE components SAC and S1PC inhibit the expression of pro-inflammatory genes (such as IL-1β, IL-6, IL-8 and G-CSF) by targeting the TLR4 receptor [172–174] and the NF-kB pathway [171,175]. A consideration should also be made concerning the CS-mediated induction of apoptosis [105–110], as outlined in chapter 3.4. Notably, Ramage et al. reported that tobacco smoke and related products induce apoptosis in A549 lung epithelial cells [105]. In this respect, reports underlining the effects of garlic compounds on induced apoptosis in several cellular model systems are available [176–179]. Finally, CS induces Reactive Oxygen Species (ROS) [112–115] and this is strongly associated with oxidative stress and human diseases [180,181]. In this respect S-allyl-l-cysteine is a strong inhibitor of ROS formation [182–185].

The industrial interest for AGE and AGE-related products is docuented by the fact that AGE is proposed and commercialized by several pharmaceutical companies, including for example Wakunaga Pharmaceuticals, Ltd (Japan) (Kyolic® Aged Garlic Extract), Evergreen Health Foods

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(Quest Kyolic Aged Garlic Extract), Shaanxi Tianrun Phytochemical Co., Ltd (Garlic Extract, Allicin), Best Pharmacy.gr (Quest Kyolic Garlic), and Bizen Chemical Co., Ltd (High SAC-Content Garlic). Notably, a Trade Mark for S-1-propenyl-l-cysteine (S1PC™) has been recently obtained by Wakunaga Pharmaceuticals (registered on July 9, 2024; https://branddb.wipo.int/; accessed on May 7th, 2025).

The industrial impact of AGE and AGE-related products is demonstrated by patents and patent application focusing on these products. For instance, US8187654B2 (Title: Process for preparing aged garlic; Assignee: Blackgarlic Inc) concerns a method of producing aged garlic in which its antioxidation capability is significantly increased as compared to that of raw garlic which is used as a raw material. Methods for preparing aged garlic are described also in US20110293803, CN110623255A, EP1752051A1, as reported by Agostinelli et al. [171].

The possible transfer of the results concerning AGE and AGE-related products from bench to the bedside is supported by the growing number pf clinical trials. For instance, NCT1950646 (The Effect of AGE on the Immune System -EAGESIS II; sponsor University of Florida; last updated 2016-02-26) demonstrated that AGE consumption modulated immune cell distribution, prevented the increase of serum TNF-$\alpha$ and IL-6 concentrations and reduced blood LDL concentration in adults with obesity [186]. A further example is NCT03860350 (Aged Garlic Extract Study – AGE; sponsor Lund University Hospital; last updated 2019-06-11) demonstrating that AGE, supplemented with B vitamins, folic acid and L-arginine retards the progression of subclinical atherosclerosis [187]. Moreover, the same NCT03860350 trial found that AGE reduced IL-6 in females with a low risk of cardiovascular diseases [188]. Relevant to this review, NCT02019368 (A Randomized, Double-blind, Placebo Controlled, Crossover Study to Evaluate the Antioxidant Effect of Aged Garlic Extract in Heavy Smokers; sponsor Hiroshima University; last updated 2015-08-19) compared the oxidative status in heavy smokers with that in non-smokers and determined the antioxidant effect of aged garlic extract (https://clinicaltrials.gov; accessed on July 18, 2025). Based on the information discussed in the present review, further clinical trials are highly warranted.

A final comment concerns the very interesting possibility that the best effects on CS induced alterations occur when natural products are employed in combination. In the study performed by Reis et al., eucalyptol and curcumin used in in combination exhibited the highest efficiency in modulating cigarette smoke extract-induced human bronchial epithelial damage [126]. Therefore, combined use of eucalyptol and curcumin might exhibit therapeutic properties against smoking-induced lung diseases [126]. Moreover, possible combinations using RNA/DNA based drugs and natural products should be in the future considered. In this respect aged garlic extract was recently proposed in combined treatments with microRNA miR-93-5p, previously demonstrated to inhibit TLR4, NF-kB and IL-8 gene expression [189]. This study provided preliminary evidence suggesting that the miR-93-5p-based miRNA therapeutics could be combined with the anti-inflammatory AGE preparation for more effectively inhibition of IL-8 gene expression [189].

## 6. Conclusions

The conclusion of this review article is that the available information strongly sustains a possible use of the anti-inflammatory aged garlic extract (AGE) and its bioactive components for mitigating the detrimental effects of cigarette smoke on human tissues. The key reasons for proposing this application are the following (summarized in Figure 3): (a) AGE and its key components are potent anti-inflammatory agents, both “in vitro” and “in vivo”; (b) the AGE bioactive components S-allyl-l-cysteine (SAC) and S1-propenyl-l-cysteine (S1PC) bind TLR4 and inhibit NF-kB; (c) AGE and AGE components SAC and S1PC inhibit the expression of pro-inflammatory genes; (d) AGE and AGE components revert apoptosis induced by cigarette smoke in several cellular model systems; (e) S-allyl-l-cysteine is a strong inhibitor of ROS formation. All the biological pathways mentioned in points (a)-(e) are strongly induced by cigarette smoke in several cellular model systems. Experimental project to verify this very interesting possibility are highly warranted, considering the impact of tobacco smoke on the health system (see Figure 1) [3–9]. It should be considered that healthcare spending attributable to cigarette smoking is very high [10,11] and that several actions have been

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considered to help quit smoking [12–15], such as bans of tobacco advertising [16] and introduction of taxes as a share of cigarette price [17,18]. These smoking cessation interventions are important [14–16,190], even if difficulty in quitting smoking might be encountered [15,16]. In this context, strategies in preventing or mitigating the effects of tobacco abuse (such as those based on natural products, including aged garlic extracts and AGE components) are of great interest, considering the world-wide distribution of tobacco abuse [1].

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## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| CS | Cigarette Smoke |
| CSC | Cigarette Smoke Condensate |
| CSE | Cigarette Smoke Extract |
| VOC | Volatile Organic Compounds |
| NF-κB | Nuclear Factor-kappa-B |
| TLR4 | Toll-like Recptor-4 |
| Nrf2 | Nuclear Factor Erythroid 2-related factor 2 |
| IL | Interleukin |
| ROS | Reactive Oxygen Species |
| AGE | Aged Garlic Extract |
| SAC | S-allyl-l-cysteine |
| S1PC | S1-propenyl-l-cysteine |
| SFN | DHS |
| COPD | Chronic Obstructive Pulmonary Disease |
| CF | Cystic Fibrosis |
| BAL | Bronchoalveolar lavage |
| WHO | World Health Organization |

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